

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference P10699 PC	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/DK 03/00812	International filing date (day/month/year) 26.11.2003	Priority date (day/month/year) 26.11.2002
International Patent Classification (IPC) or both national classification and IPC A61K47/48		
Applicant DANMARKS FODEVARE- OG VETERINAERFORSKNING et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 9 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 29.06.2004	Date of completion of this report 04.03.2005
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**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. **PCT/DK 03/00812**

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, Pages

1-26 as published

Claims, Numbers

1-46 received on 14.02.2005 with letter of 11.02.2005

Drawings, Sheets

1/3-3/3 as published

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

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5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).
- (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:
- ☐ the entire international application,
 - ☒ claims Nos. 19-23,27-32,35,38
because:
 - ☒ the said international application, or the said claims Nos. 19-23,27-32,35,38 relate to the following subject matter which does not require an international preliminary examination (specify):
see separate sheet
 - ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):
 - ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.
 - ☐ no international search report has been established for the said claims Nos.
2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:
- ☐ the written form has not been furnished or does not comply with the Standard.
 - ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	1-46
	No: Claims	
Inventive step (IS)	Yes: Claims	1-46
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-18,24-26,33,34,36,37,39-46
	No: Claims	

2. Citations and explanations

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see separate sheet

Basis of the report

Reference is made to the following documents:

D2: WO 00/72851 A (SCOTT M R et al.) 7 December 2000;

D3: WO 01/54736 A (SCOTT M R et al.) 2 August 2001;

D4: SUPATTAPONE S et al.: "Elimination of prions by branched polyamines and implications for therapeutics." Proceedings of the National Academy of Sciences of the United States of America, vol. 96, no. 25, 7 December 1999, pages 14529-14534, XP0002186756 ISSN: 0027-8424;

D6: US-A-4 587 329 (DEWALD JAMES R ET AL) 6 May 1986 (1986-05-06).

The application discloses (the references in parentheses applying to this document) a dendrimer conjugate having the structure $D(R)_n$, as defined in claim 1 (claims 1-18).

The application discloses as well several uses of said dendrimer conjugate (Claims 19-39), and several methods for the preparation of said dendrimer conjugate (claims 40-46).

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

For the assessment of the present claims 19-23, 27-32, 35 and 38 on the question whether they are industrially applicable, no unified criteria exists in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognise as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims

to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment (Article 34(4)(a)(i) PCT, see also the PCT-guidelines IV-2.4.(d) and IV-2.5).
Nevertheless, an international preliminary examination on novelty and inventive step of the subject-matter of the above-mentioned claims is being carried out with respect to the alleged effects underlying said uses.

Re Item V

**Reasoned statement with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1 Prior art:

The document **D1** discloses (the references in parentheses applying to this document) branched polyamines, including polyamidoamine and polypropyleneimine (PPI) dendrimers, which are able to purge PrP^{Sc}, the disease-causing isoform of the prion protein, from scrapie-infected neuroblastoma (ScN2a) cells in culture. Branched polyamines are the first class of compounds shown to cure prion infection in living cells and may prove useful as therapeutic, disinfecting, and strain-typing reagents for prion diseases (Abstract).

The document **D2** discloses (the references in parentheses applying to this document) a method of sterilizing objects as well as the sterilized objects obtained from the method are disclosed. The method involves contacting an object such as a medical device to be reused with a polycationic dendrimer under conditions which result in rendering a conformationally altered protein (e.g. a prion) non-infectious (Abstract). The polycationic dendrimer is of structure D(R)_n, wherein D is of formula $[-(N-CH_2-CH_2-NH-CO-CH_2-CH_2)_2]NCH_2-CH_2-N[(CH_2-CH_2-CO-NH-CH_2-CH_2-N)_2]$, and R is of formula $-CH_2-CH_2-CO-NH-CH_2-CH_2-N(CH_2-CH_2-CO-NH-CH_2-CH_2-NH_2)_2$, which is different from D (Fig 1).

The document **D3** discloses (the references in parentheses applying to this document) an antiseptic composition comprising solvent, an acid component for maintaining the pH of the composition at at most 5, and an active component for reducing infectivity of an

infectious protein when the composition is brought into contact with the infectious protein for at most 2 hours (claim 1). The active component can be a dendrimer (of the structure $D(R)_n$, as the dendrimer disclosed in **D2**, wherein R is different from D) or a protein denaturant (claims 8, 13, 21); and said dendrimer can be used *in vivo* as a therapeutic agent (page 50, lines 5-26).

The document **D6** discloses (the references in parentheses applying to this document) a dendrimer conjugate of a dendrimer and a carboxylic acid ester (col 19, formula), or a dendrimer conjugate of formula $D(R)_2$, wherein D is of formula $(Ph)(CH_2-S-CH_2-CH_2-N)_6$, and R is of formula $CH_2CH_2N(CH_2CH_2CO-NH-CH_2CH_2NH)_2$ which R is different from D (col 28, line 30). In preferred dense star polymers, the terminal groups are functional groups (such as urea) that are sufficiently reactive to undergo addition or substitution reactions (col 6, lines 12-19).

None of said documents **D1**, **D2**, **D3** or **D6** discloses or suggests a dendrimer conjugate according to claim 1 of the application.

2 Claims 1-18:

The document **D4** is regarded as being the closest prior art to the subject-matter of claim 1, and discloses (the references in parentheses applying to this document) branched polyamines, including polyamidoamide dendrimers, polypropyleneimine, and polyethyleneimine (Table 1, Fig 2), which are able to purge PrP^{Sc} , the protease-resistant isoform of the prion protein, from scrapie-infected neuroblastoma (ScN2a) cells in culture (Abstract).

The subject-matter of claim 1 **differs** from this known dendrimer conjugate in the structure of R (which comprises a group $-NHY$).

The subject-matter of **claims 1-18** can therefore be considered **new** (Article 33(2) PCT).

The **problem** to be solved by the present invention may therefore be regarded as to provide alternative dendrimer conjugates.

The **solution** proposed in claim 1 of the present application can be considered as involving an inventive step (Article 33(3) PCT), because a dendrimer conjugate wherein R comprises a group -NHY as defined in claim 1 is not disclosed or suggested by the available prior art documents.

The subject-matter of **claims 1-18** can therefore be considered as involving an **inventive** step (Article 33(3) PCT).

3 Claims 19-46

The use of a new and inventive compound, and a method for the preparation of a new and inventive compound can be considered new and inventive.

Therefore, the subject-matter of **claims 19-46** **complies** with the requirements of Articles 33(2) and 33(3) PCT.

4 A dendrimer conjugate according to claim 1 has an application e.g in the preparation of a medicament for use in the treatment, prophylaxis and/or diagnosis of protein aggregate related diseases.

Therefore, the subject-matter of **claims 1-18, 24-26, 33, 34, 36, 37 and 39-46** **complies** with the requirements of Article 33(4) PCT.

5 **Certain observations on the international application**

5.1 Claims 1 and 18 contain references to the description ("by a protease assay as described herein"; "where EC50 is as defined herein"). According to Rule 6.2(a) PCT, claims should not contain such references. Moreover the expressions "protease assay" and "EC50" used in said claims are vague and unclear and leave the reader in doubt as to the meaning of the technical features to which they refer, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

5.2 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document **D4** is not mentioned in the description, nor is this document identified therein.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/DK 03/00812

- 5.3 There are spelling mistakes in the application:
claim 17: "as defined in claims 10-18";
claim 21: "Huntingtin"

CLAIMS

1. A dendrimer conjugate formed between a dendrimer and a protein solubilising substance, said protein solubilising substance having a structure which is not found in the dendrimer, and the conjugate – upon treatment of protein aggregates with the dendrimer conjugate – causing an increase in the solubility of protein aggregates over that obtained upon treatment of protein aggregates under the same treatment conditions with a physical mixture of the dendrimer and protein solubilising substance, the physical mixture containing the same molar ratio of the protein solubilising substance to the dendrimer as that in the dendrimer conjugate, and the increase being evidenced by a protease assay as described herein.

2. A dendrimer conjugate according to claim 1, wherein the dendrimer is covalently bound to one or more same or different protein solubilising substances.

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3. A dendrimer conjugate according to claim 1 or 2 having the structure



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wherein

D is the dendrimer

R is a radical of the protein solubilising substance which may be the same or different, and

25 n is an integer greater than 1.

4. A dendrimer conjugate according to claim 3, wherein R is bound to the surface groups of the dendrimer.

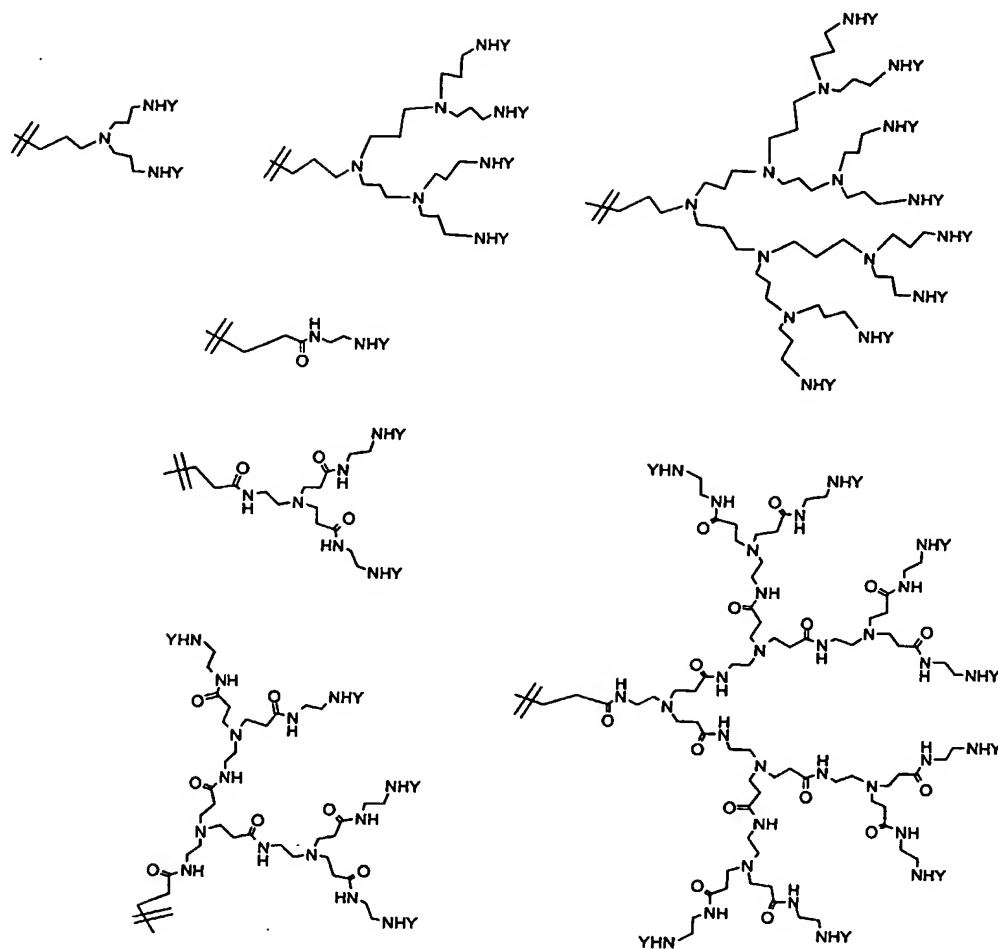
30 5. A dendrimer conjugate according to any of claims 1-4, wherein the protein solubilising substance is a protein denaturant.

6. A dendrimer conjugate according to claim 5, wherein the protein denaturant is selected from the group consisting of ureas, thioureas, sulfonylureas, semicarbazides, hydrazides, thiosemicarbazides, guanidines and chaotropes.

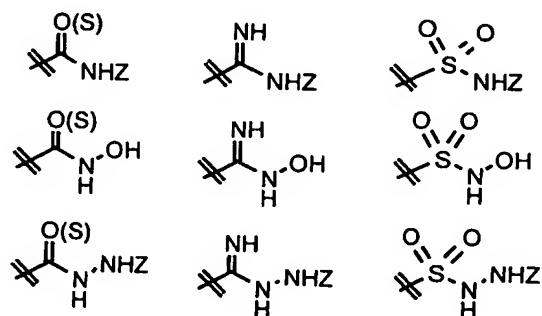
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7. A dendrimer conjugate according to claim 3, wherein R is

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ART 34 AMDT**

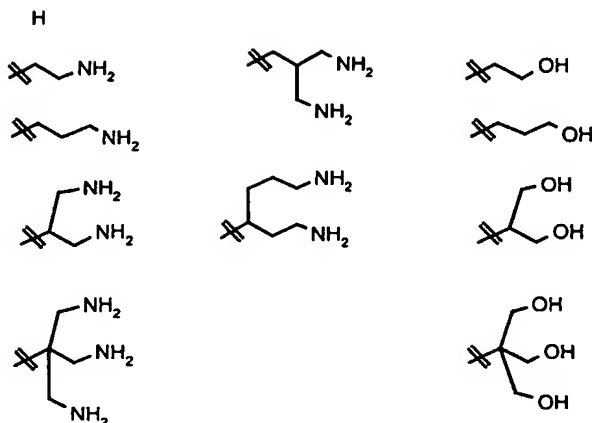


5 wherein Y is selected from the group consisting of



wherein Z is selected from the group consisting of

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8. A dendrimer conjugate according to any of claims 1-7, wherein the solubility of the protein aggregates is increased by a factor of more than 1 such as, e.g., at least 1.5 or at least 2.

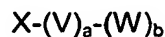
9. A dendrimer conjugate according to any of claims 1-8 containing one or more surface groups which are not occupied by a protein solubilising substance

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10. A dendrimer conjugate according to any of claims 1-9, wherein the dendrimer (D) is a multivalent functional dendrimer having a dendritic structure that extends from one or more core points through multiple generations of successive layers, with each layer having one or more branching points, to end in surface groups.

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11. A dendrimer conjugate according to claim 10, wherein the dendrimer (D) is represented by the formula:



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wherein X is a multifunctional segment having one or more branching points,

V is a linker or spacer group, which may be branched or linear

W is a surface group and

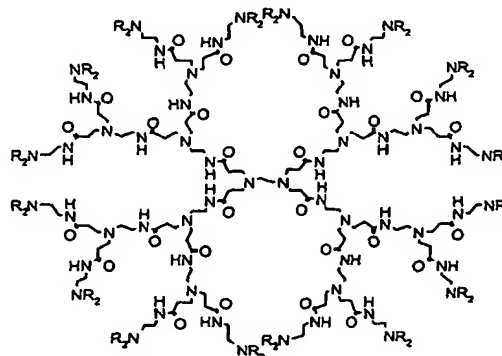
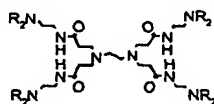
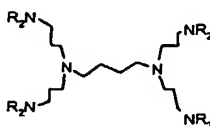
a and b are integers such that each linker group V terminates in one or more surface groups W.

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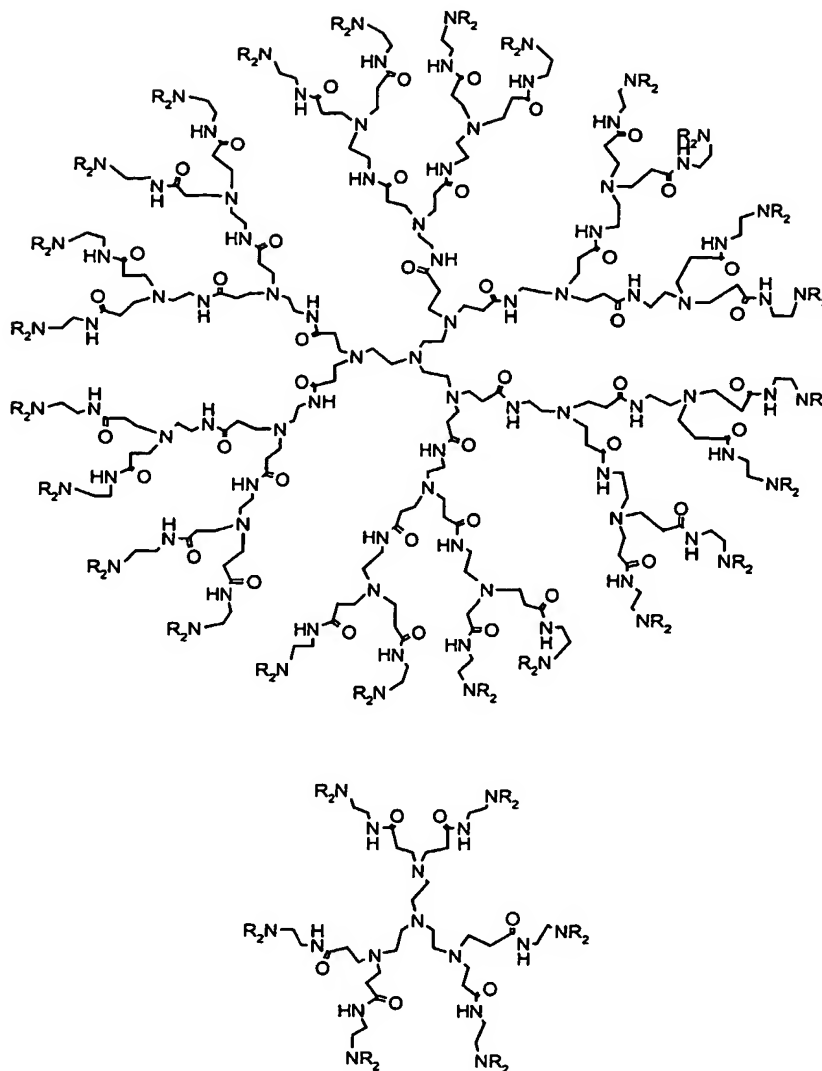
12. A dendrimer conjugate according to any of claims 10 or 11, wherein the dendrimer is globular or tree-shaped.

REPLACED BY
ART 34 AMDT

13. A dendrimer conjugate according to any of claims 10-12, wherein the generation of the dendrimer ranges from 0 to 20 such as e.g. from 1 to 10 or from 2 to 6.
14. A dendrimer conjugate according to any of claims 10-13, wherein the molecular mass of the unmodified dendrimer lies from 50 to 30000 such as e.g. from 100 to 20000 or from 300 to 15000.
15. A dendrimer conjugate according to any of claims 10-14, wherein the number of surface groups on the dendrimer lies between 2 and 256 such as e.g. between 2 and 64, between 4 and 32 or between 8 and 32, such as e.g. 4, 8, 16, 32 or 64.
16. A dendrimer conjugate according to any of claims 10-15, wherein the surface groups of the dendrimer (D) are amine functionalities.
17. A dendrimer conjugate according to any of claims 10-16, wherein the dendrimer is a PPI dendrimer or a PEI dendrimer or a PAMAM dendrimer.
18. A dendrimer conjugate according to any of claims 10-17, wherein the dendrimer has one of the following structures



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5 wherein R has the same meaning as in claim 3.

19. A dendrimer conjugate according to any of claims 1-9, wherein the dendrimer (D) is a conjugate of two or more multivalent functional dendrimers as defined in claims 10-18.

10 20. A dendrimer conjugate according to any of the preceding claims, which has an EC₅₀ value of 10-500 μ g/ml, such as e.g. 20-200 μ g/ml, 30-100 μ g/ml or 50 μ g/ml, where EC₅₀ is as defined herein.

15 21. Use of a dendrimer conjugate according to any of claims 1-20 in the treatment of protein aggregate related diseases.

22. Use according to claim 21, wherein the protein aggregate related disease is

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selected from the group consisting of Alzheimer's disease, Creutzfeldt-Jakob disease, variant Creutzfeldt-Jakobs disease, fatal familial insomnia, Gerstmann-Sträussler-Sheinker syndrome, PrP-cerebral amyloid angiopathy, scrapie, bovine spongiform encephalopathy, chronic wasting disease, transmissible mink encephalopathy, Pick's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia, diabetes type II, multiple myeloma-plasma cell dyscrasias, familial amyloidotic polyneuropathy, medullary carcinoma of thyroid, chronic renal failure, congestive heart failure, senile cardiac and systemic amyloidosis, chronic inflammation, atherosclerosis, familial amyloidosis and Huntington's disease.

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23. Use according to any of claims 21 or 22, wherein the protein of the protein aggregate is selected from the group consisting of APP, A β peptide, α 1-antichymotrypsin, tau, non-A β -component, presenillin 1, presenillin 2, apoE, prion protein including protease resistant prion protein, SOD, Pick body, α -synuclein, anylin, IgGL-chain, transthyretin, procalcitonin, β 2-microglobulin, atrial natriuretic factor, serum amyloid A, ApoA1, Gelsolin and Huntingtin.

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24. Use of dendrimer conjugates according to any of claims 21 or 22, wherein the protein aggregate related disease is a prion-related disease.

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25. Use of dendrimer conjugates according to any of claims 21 or 22, wherein the protein aggregate related disease is an amyloid-related disease.

26. Use of dendrimer conjugates according to any of claims 1-20 to reduce the infectivity of prion proteins.

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27. Use of dendrimer conjugates according to any of claims 1-20 in disinfection of material which has been contaminated with protein aggregates.

28. Use of dendrimer conjugates according to claim 27, wherein the protein aggregates are prion protein aggregates.

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29. A method of identifying and/or classifying protein aggregates in a mammal, the method comprising the steps of:

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- a) treating the protein aggregates with a dendrimer conjugate as defined in claims 1-20

b) analysing one or more products of step a)

30. A method according to claim 29 wherein step b) comprises the steps of

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I. incubating the treated protein aggregates from step a) with a broad spectrum protease such as e.g. proteinase K

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II. detecting remaining protein aggregates by one or more methods selected from the group comprising: SDS-PAGE and immunoblotting with protein-specific antibodies, ELISA, immunoelectrophoresis and immunohistochemistry.

31. A method according to claim 29 wherein step b) comprises incubating the treated protein aggregates from step a) with an antibody which is sensitive to changes in the structure of a protein present in the protein aggregate.

32. A method according to claim 29 additionally comprising the step of further treating the treated protein aggregates from step a) with a protein denaturant such as e.g. urea between steps a) and b).

33. A method according to claim 29 further comprising the steps of

25 i) repeating steps a) and b) with a different dendrimer conjugate, and
ii) optionally comparing results from the dendrimer conjugates to obtain information on the origin of the protein aggregates.

34. Use of dendrimer conjugates according to any of claims 1-20 for identifying prion protein aggregates.

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35. Use of dendrimer conjugates according to any of claims 1-20 for classifying the protein aggregates into specific strains according to their susceptibility to the method described in claim 29.

35 36. Use of dendrimer conjugates according to claim 35, wherein the protein aggregates are prion protein aggregates.

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37. A method of preventing the formation of protein aggregates in cells or animals, the method comprising the treatment of cells or animals with a dendrimer conjugate according to any of claims 1-20.
- 5 38. A method for disinfecting an object, the method comprising contacting the object with a composition containing a dendrimer conjugate according to any of claims 1-20.
39. A method for removing protein aggregates from food that originates from an animal, the method comprising contacting the food with a composition containing a
10 dendrimer conjugate according to any of claims 1-20.
40. A method for treating, preventing and/or diagnosing a protein aggregate related disease in a subject, the method comprising administering to the subject in need thereof a sufficient amount of a dendrimer conjugate according to any of claims 1-20.
- 15 41. Use of dendrimer conjugates according to any of claims 1-20 in the preparation of a medicament for use in the treatment, prophylaxis and/or diagnosis of protein aggregate related diseases.
- 20 42. A method for the preparation of a dendrimer conjugate according to any of claims 1-20 wherein the preparation is carried out while the dendrimer (D) is grafted to a solid phase support through a linker entity.
43. A method according to claim 42 wherein the linker entity is an acid labile linker,
25 such as e.g. chlorotriylchloride, Wang, Rink, Sieber or related linkers.
44. A method according to claim 43 wherein the solid phase support is selected from the group comprising polystyrene, modified polystyrene and PEGA.
- 30 45. A method for the preparation of a dendrimer conjugate according to any of claims 1-20 in which the dendrimer conjugate contains surface sulfonylurea (sulfamide) groups, the method comprising the reaction of dendrimer with a sulfonylamide ($>SO_2NHR$) reagent, such as e.g. chlorosulfonyl-isocyanate, halo-sulfamide, chlorsulfonyl-*tert*-butylsulfamate or other sulfonylamide reagent.
- 35 46. A method for the preparation of a dendrimer conjugate according to any of claims 1-20 in which the dendrimer conjugate contains surface guanidine groups, the method

comprising the reaction of dendrimer with di-boc-S-methylisothiourea, di-Boc-thiourea or condensing agents such as carbodiimides, phosphonium salts or other condensing reagents.

- 5 47. A method for the preparation of a dendrimer conjugate according to any of claims 1-20 in which the dendrimer conjugate contains surface thiourea groups, the method comprising the reaction of dendrimer with thiocarbamoyl $-(C=S)NHR$ reagents, such as e.g. alkyl-thiocarbamoyl halides or other thiocarbamoyl reagents.
- 10 48. A method for the preparation of a dendrimer conjugate according to any of claims 1-20 in which the dendrimer conjugate contains surface urea groups, the method comprising the reaction of dendrimer with carbamoyl $-(C=O)NHR$ reagents such as e.g. alkyl-carbamoyl halides or other carbamoyl reagents.

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ART 34 AMDT